EXHIBIT 2

Digoxin Toxicity Statement
Marc J. Semigran MD

The digitalis glycosides are a group of steroid-based compounds that exert characteristic positive inotropic and electrophysiological effects on the heart. This series of compounds contains a steroid nucleus that is linked to a lactone ring at carbon 17 and series of sugars at carbon 3 (Figure 1). Over 300 cardiac glycoside compounds have been described that exert cardiac effects; by far the agent most commonly prescribed in the United States is digoxin. Digoxin itself consists of 3 digitoxose sugar moieties bound to the digoxigenin steroid nucleus. The sugar moieties determine the rate of absorption and kinetics of action of the drug, while the steroid nucleus, often referred to as the aglycone, determines the cardiac activity.

Cardiac glycosides have played an important role in the treatment of heart failure since William Withering described their use in a classic monograph on the efficacy of the leaves of the foxglove plant in 1785. From that time through much of the twentieth century, digitalis preparations were the mainstay of heart failure treatment, with improvement in edema and breathlessness that could be quite dramatic. The utility of digoxin therapy for heart failure treatment, particularly in regards to altering patient outcomes such as need for hospitalization and survival, has more recently come under scrutiny. This is related to the development of other, less-toxic, treatments for heart failure, such as the loop diuretics, vasodilators, and neurohormonal antagonists such as angiotensin-converting enzyme inhibitors, β-adrenergic antagonists, and aldosterone-receptor antagonists. In addition, controversy remains about the role of digoxin therapy in certain patient subgroups, in particular those with normal left ventricular systolic function. The debate regarding the benefits of digoxin therapy is particularly important when contrasted with the frequency of digoxin toxicity, which is related to the occurrence of toxic effects at plasma levels that are in the upper range of what is considered therapeutic.

Mechanism of action – Inotropic effect

Ex vivo studies of changes in cardiac pressure and volume in intact preparations of canine hearts during the 19020's led to the conclusion that cardiac glycosides caused a positive inotropic effect. It was observed in both normal hearts and those from animal models of heart failure that cardiac glycoside administration resulted in an increase in the rate of change of ventricular pressure during isovolumic contraction (peak +dP/dt). Further studies of preparations of digitalis preparations administered to isolated hearts contracting in an apparatus in which cardiac filling (preload) and outflow pressure (afterload) were performed in the 18050's and 1960's. It was observed that digoxin led to a shift of the ventricular function (Frank-Starling) curve of stroke work vs. filling pressure, indicative of an increase in myocardial contractility. The positive inotropic effect was observed to

be dependent on heart rate, with a peak effect at heart rates between 75-100 min^{-1}, and declining at both higher and lower rates.

Further studies cardiac muscle using Na+ sensitive microelectrodes and intracellular probes of calcium ion fluxes in cardiac myocytes have demonstrated that the positive inotropic effect of digoxin is mediated by increased intracellular levels of calcium. This increase in intracellular calcium is a manifestation of the inhibitory effect of digoxin on transmembrane Na+ transport.

Cardiac glycosides, including digoxin, are potent and specific inhibitors of the membrane-bound enzyme sodium, potassium-adenosine triphosphatase (Na^+-K^+ ATPase). This enzyme has been highly conserved throughout eukaryotes for the maintenance of transmembrane cellular Na+ and K+ gradients. The enzyme is present in the membranes of cardiac cells (including the specialized cardiac cells responsible for conduction of the electrical impulse that regulates synchronicity of contraction), vascular smooth muscle cells, and neurons. The enzyme is composed of multiple subunits, one of which is the α-subunit that contains on its extracytoplasmic face a binding site for cardiac glycosides. Binding of a molecule to this binding site results in the complete inhibition of the enzymatic and transport function of the Na^+-K^+ ATPase. Optimal binding of the inhibitory glycoside to the α subunit requires the presence of Na+, Mg2+, and ATP. Of clinical relevance is the observation that glycoside binding and enzyme inhibition is itself inhibited by extracellular K+.

With each depolarizing action potential, the electrical signal that stimulates cardiomyocyte contraction, membrane channels open permitting an influx of Na+ into the cell. The usual response of the cell, aiming to restore the resting transmembrane potential, would be to pump Na+ back out of the cell using the Na+, K+ ATPase. In the presence of digoxin, the Na+, K+ ATPase is inhibited and the majority of Na+ must be transported out of the cell via another transmembrane protein, the Na+, Ca++ exchanger. This exchanger allows Na+ efflux to occur simultaneously with Ca++ influx, with the concentration gradient driving Ca++ into the cytoplasm. The intracellular calcium then stimulates cardiac contraction through binding to troponin C permitting actin and myosin to interact and generate force. With inhibition of Na+, K+ ATPase, a greater fraction of Na+ is extruded from the cell via the Na+, Ca++ exchanger, leading to a higher intracellular Ca++ with each action potential, and greater contractile force.

Mechanism of action – Electrophysiological effects

Similar to the inotropic effect of cardiac glycosides, the electrophysiological effect of these agents is based on inhibition of the Na+, K+ ATPase. The persistent reliance on the Na+, Ca++ transmembrane ion exchanger to restore the resting transmembrane potential of -80-90 mV is less effective than the inhibited Na+, K+ ATPase and in combination with increased Ca++ the magnitude of the resting

potential is reduced. This slows the rate of cardiac cell depolarization and action potential duration, leading to overall decreased conduction of the action potential through the heart. This slowing of conduction velocity, when occurring in the atrioventricular node, can lead to a slowing of the ventricular rate during atrial fibrillation and other supraventricular tachycardias.

The electrophysiological effects of digoxin may be most manifest when toxic levels of the agent are present. In this situation, it is believed that the increased intracellular calcium concentration exceeds the capacity of calsequestrin in the sarcoplasmic reticulum to retain calcium. The overload leads to spontaneous cycles of Ca++ release and reuptake by the cell through poorly regulated, non-specific cation channels. Dispersion of refractory periods in the myocardium results, serving as the substrate for the ventricular arrhythmias observed with digoxin toxicity.

Neurohormonal effects of digoxin

Direct recordings of sympathetic nervous activity in both animal models of heart failure and patients have shown that cardiac glycosides can directly stimulate baroreceptors resulting in inhibition of sympathetic outflow. This can directly lead to the beneficial slowing of heart rate observed with digoxin treatment of patients with atrial arrhythmia. In addition, the inhibition of sympathetic outflow may be the source of the decreased levels of serum norepinephrine, plasma renin activity, and aldosterone seen in heart failure patients treated with digoxin. In fact, these may be the most significant beneficial effects of digoxin therapy of heart failure, and they appear to occur at plasma levels significantly below those necessary to achieve direct inotropic effects (1).

Clinical Use of Digoxin

Heart Failure

Although digoxin has been used to treat heart failure for over 200 years, it was not until the mid twentieth century that clinical studies demonstrated its utility in heart failure patients with normal sinus rhythm. The firm establishment of digoxin therapy as a treatment for heart failure based on its long history of use made the conduct of placebo-controlled studies difficult to perform in the modern era of evidence-based medicine due to lack of acceptance by clinicians of a placebo control arm.

Overall, modern studies suggest a beneficial effect of digoxin therapy in more severely ill heart failure patients with left ventricular systolic dysfunction. Specifically, a landmark study in 1982 performed by the Massachusetts General Hospital Medical House Staff observed that digoxin had beneficial effects on symptoms in heart failure patients only if a third heart sound was present (2). Other studies have shown beneficial effects on cardiac performance in heart

failure patients who remained symptomatic after treatment with diuretics and vasodilators, but not in those patients that became compensated after these treatments. More recently, the captopril-digoxin trial of NYHA Class I and II patients showed similar benefits on the need for increased diuretic doses or hospitalization of either digoxin or captopril therapy as compared to placebo (3), and the milrinone-digoxin trial observed that digoxin therapy improved exercise capacity and the frequency of decompensation of moderately severely-ill heart failure patients compared with placebo (4).

In 1993, two trials, the Prospective Randomized Study of Ventricular Failure and Efficacy of Digoxin (PROVED) and the Randomized Assessment of Digoxin on Inhibitors of Angiotensin-Converting-Enzyme (RADIANCE), examined the effects of withdrawal of digoxin in patients with stable, mild to moderate heart failure and left ventricular systolic dysfunction. In both trials, while patients experienced less clinical deterioration and hospitalizations, their survival benefit was not increased. In 1997, the Digitalis Investigation Group study again showed that patients on digoxin with CHF had fewer hospitalizations for heart failure.

Current Guideline Recommendations for the Use of Digoxin to Treat Heart Failure Patients (5)

The most recent American Heart Association/American College of Cardiology guidelines for the treatment of heart failure recommend the use of digoxin as a "fourth" agent in symptomatic heart failure patients to reduce symptoms, prevent hospitalization, control rhythm, and enhance exercise tolerance. The Guidelines recommend against the use of digoxin in asymptomatic patients, even with the presence of a low LVEF.

Due to the risk of toxicity of digoxin, current Guidelines suggest the use of low doses of digoxin in heart failure patients, to "achieve a concentration of drug in plasma in the range of 0.5 to 1.0 ng per mL." This recommendation is based on retrospective analysis of both the PROVED and RADIANCE studies of digoxin withdrawal that the prevention of worsening HF by digoxin at lower concentrations in plasma (0.5 to 0.9 ng/mL) was as great as that achieved at higher concentrations. Furthermore, in a retrospective analysis of the Digitalis Investigation Group trial, risk-adjusted mortality increased as the plasma concentrations exceeded 1.0 ng per mL.

Digoxin Toxicity

Epidemiology

Digoxin toxicity remains one of the most prevalent adverse drug reactions seen in clinical practice, likely because of the relatively small toxic/therapeutic ratio of plasma levels. The incidence of cardiac toxicity in retrospective studies of hospitalized patients in the mid-twentieth century ranged from 8 to 23%. It is

believed that the subsequent increased awareness of drug interactions with digoxin and the availability of accurate rapid radioimmunoassays to monitor drug levels have lead to a decline in the incidence of digoxin toxicity. Also of importance in the reduction of digoxin toxicity is the recent development of formulations with more predictable drug availability (6), highlighting the increased risk of toxicity in patients taking digoxin manufactured with less accurate amounts of drug.

However, in the recent prospective DIG trial, where patients were carefully monitored, 12% of the patients in the treatment arm were suspected of having digoxin toxicity (7). Atrial and ventricular arrhythmias were the most frequent reasons for the suspicion of digoxin toxicity in that study, and remain the major concern of physicians dealing with this problem. Reported mortality of digoxin toxicity is high, despite the availability of treatments, with 22 deaths reported to the US Poison Control Center in 2006 (8), and 10 deaths in 2007 (9). These figures likely represent a marked underestimate, as many episodes of digoxin toxicity are not reported to the Center. Overall mortality from an episode of digoxin toxicity in series of hospitalized patients ranges from 5-10%. Older patients, and those with the most severe heart failure, are at greatest risk for an adverse outcome during an episode of digoxin toxicity.

Factors leading to or contributing to digoxin toxicity

A number of factors that have even a most effect on digoxin levels can lead to digoxin toxicity because of the relatively narrow difference between concentrations that are therapeutic and those that are toxic. Certainly factors that alter the amount of drug absorbed and excreted are among the most common causes of digoxin toxicity. Small changes in the amount of drug in the formulation, or its bioavailability, can lead to clinically significant changes in plasma levels. A decrease in renal function, related to either intrinsic renal disease or pre-renal factors such as dehydration or diminished cardiac output, can lead to diminished digoxin clearance and toxicity.

Drug interactions with digoxin can occur, and because they lead to alterations in digoxin absorption, plasma protein binding and hence availability, electrophysiological effects, and clearance, can cause digoxin toxicity. Among the more commonly observed clinical drug interactions with digoxin that lead to toxicity are:

- Amiodarone - Reduces renal and nonrenal clearance of digoxin and may have additive effects on the heart rate,
- Carvedilol - May increase digoxin blood levels in addition to additive effects on slowing heart rate
- Calcium channel blockers - Diltiazem and verapamil increase serum digoxin levels; not all calcium channel blockers share this effect.

- Cyclosporine - May increase digoxin levels, possibly due to reduced renal excretion
- Erythromycin, clarithromycin, and tetracyclines - May increase digoxin levels
- Propafenone - Increases digoxin levels
- Quinidine - Increases digoxin level as much as two-fold
- Rifampin – Decreases digoxin levels by increasing metabolism; hence cessation of rifampin can lead to an increase in digoxin level.

Myocardial Ischemia and infarction

Myocardial ischemia inhibits the Na+, K+ ATPase that is the target of digoxin and can thus contribute to toxicity. In addition, ischemia increases myocardial automaticity that can augment the proarrhythmic effect of digoxin toxicity. Thus, the occurrence of elevated digoxin levels in the setting of either ischemic heart disease or an acute coronary syndrome can have an even greater propensity to toxic arrhythmias.

Symptoms and signs of digoxin toxicity

Symptoms of cardiac glycoside toxicity are mostly nonspecific, and include fatigue, blurred vision, disturbed color perception, anorexia, nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, confusion, delirium, and occasionally hallucinations. Most common are the gastrointestinal symptoms, and visual changes such as the appearance of a yellow-green coloring to objects and a halo surrounding objects. Objective findings of digoxin toxicity on physical exam are rare. Despite the symptoms, the toxic patient's abdominal and neurological exams are usually benign, with a change in mental status being observed with high digoxin levels. Despite the visual symptoms, the ocular exam is benign.

Electrocardiographic manifestations of digoxin toxicity

The electrocardiogram is altered by even therapeutic levels of digoxin, with the characteristic changes being a "scooping" of the ST segment and prolongation of the PR interval. With the development of toxicity, arrhythmias occur, characterized by either slowing of conduction or the increased automaticity of subsidiary or ectopic cardiac pacemakers. These include:

1. Atrioventricular junctional escape rhythms
2. Ventricular bigeminy or trigeminy
3. Nonparoxysmal atrioventricular junctional tachycardia with high degrees of block (4:1, 6:1)
4. Ectopic ventricular beats (usually >5/min)
5. Multifocal ectopic ventricular beats (can be $\leq$ 5/min)
6. Ventricular tachycardia
7. Ventricular fibrillation

8. Paroxysmal atrial tachycardia with atrioventricular block (high degree)
9. Sinus arrest or sinoatrial exit block
10. Mobitz I second degree atrioventricular block
11. Third degree (complete) heart block
12. Asystole

Digoxin toxicity is also one of the few causes of arrhythmia characterized by combinations of increased automaticity and slowed conduction, such as the "double tachycardia" of atrioventricular dissociation with simultaneous atrial and junctional tachycardia, or atrial fibrillation with regularization of the ventricular response due to an accelerated junctional or ventricular pacemaker focus.

As asystole and ventricular fibrillation can be the arrhythmic manifestations of digoxin toxicity, patients can present with sudden death without any premonitory arrhythmia.

Laboratory Findings

Serum potassium is important in both the diagnosis and management of digoxin toxicity. Digoxin inhibits the Na+, K+ ATPase, which normally transports potassium into the cell against a concentration gradient. Thus high levels of serum potassium can be a marker of digoxin toxicity, and they are also prognostic. In a study performed prior to the easy availability of therapeutic digoxin binding antibody, patients presenting with potassium levels above 5.5 meq/dL had a 100 percent mortality (10).

On the other hand, hypokalemia can contribute to the toxic effects of digoxin. Potassium is a substrate for Na+, K+ ATPase, and low concentrations of potassium will decrease enzyme activity and exacerbate the intracellular elevation of sodium ion concentration and its effects on calcium fluxes and the electrical properties of the cardiomyocyte. Hypomagnesemia can also facilitate digoxin toxic arrhythmias. The presence of these electrolyte abnormalities is not uncommon in heart failure patients that may be taking digoxin, as diuretic therapy that is commonly used in their treatment can lead to renal loss of potassium and magnesium.

Hypercalcemia can also exacerbate the toxic effects of a given level of digoxin, as it leads to increased intracellular calcium levels through the membrane sodium-calcium exchanger.

Acidosis depresses the Na+, K+ ATPase pump and may cause the effects of digoxin toxicity to be more manifest at a given plasma digoxin level. The presence of renal insufficiency, because it may both decrease digoxin clearance and contribute to acidosis, can significantly contribute to digoxin toxicity.

Plasma digoxin levels

Plasma digoxin levels are a marker of tissue levels, and hence of the cellular effects of digoxin. Time is required for both absorption and distribution of a dose of digoxin; hence, if the level is drawn too soon after ingestion, it may not reflect the eventual tissue levels of the dose. On the other hand, levels that are drawn after a dose is absorbed, but not yet equilibrated into the eventual volume of tissue distribution, may be higher than is representative of the cellular effect.

Endogenous digoxin-like substances can be immunoreactive and hence lead to the measurement of elevated plasma digoxin levels that are either falsely elevated or falsely depressed. These endogenous substances have been observed to be present in infants and in pregnant women, and in patients with liver or biliary disease and in those with chronic kidney disease. Chinese herbal medicines have been reported to interfere with the digoxin assay, with varying effects depending on the assay used (11). Because of the specificity of the assay for digoxin, other cardiac glycosides, such as those directly present in plant leaves, will not be measured in the assay.

A plasma digoxin level of greater than 2.0 ng/ml is considered toxic, though as noted above, the presence of ischemic heart disease, hypokalemia, or the other metabolic abnormalities cited above can lead to toxic effects at lower levels. Current guidelines for the treatment of heart failure recommend that patients treated chronically with digoxin have plasma levels of 0.5-0.9 ng/ml (5).

Treatment

The treatment of digoxin toxicity should focus on supportive care of any respiratory, hemodynamic, or electrophysiological manifestations of the toxicity, an effort to correct any metabolic abnormalities that can contribute to hemodynamic instability and the removal of digoxin from the body, either by preventing its absorption from the gastrointestinal system, or removing it from the bloodstream and tissues.

Patients that are unable to protect their airway because of mental status changes resulting form digoxin toxicity should be intubated, and hypoxia should be treated with supplemental oxygen. Significant bradyarrhythmias should be treated with atropine. A temporary pacemaker may be required in patients with persistent bradyarrhythmia. The use of catecholamines to increase heart rate is discouraged because of their own propensity to increase automaticity and thus induce potentially lethal tachyarrhythmias. Lidocaine and phenytoin are believed to be the optimal agents to treat ventricular tachycardia. Phenytoin in particular is desirable, as it can decrease cardiac myocyte automaticity without slowing atrioventricular conduction. Adrenergic antagonists such as β-receptor blockers are a second choice. They can reduce tachyarrhythmias, but also induce atrioventricular block.

Both digoxin and its metabolites that are excreted through the biliary circulation are adsorbed by activated charcoal, and it should be administered to digoxin toxic patients that have recently ingested the drug. The gastric lavage often necessary to administer activated charcoal and to remove undigested medication increases vagal tone and may precipitate or worsen arrhythmias.

As mentioned above, electrolyte abnormalities such as hypokalemia and hypomagnesemia can exacerbate the toxic effects of digoxin, and should be promptly treated. Only moderately severe hyperkalemia should be treated (K+> 5.5 meq/L) so as to avoid any adverse effects of transient lowering of serum potassium.

Digoxin-specific antibodies Digoxin-specific antibody Fab fragments are purified from the immunoglobulin of sheep that are immunized with digoxin. They avidly bind to intravascular digoxin and the complex, including the digoxin molecule, is excreted by the kidneys. The Fab fragments can diffuse into the interstitial space where they bind to digoxin in the tissues. As a result, the free digoxin concentration in the interstitial space is markedly reduced, creating a favorable concentration gradient for the efflux of digoxin out of the cells and into the extracellular fluid where it binds to digoxin-specific antibody fragments. Bound digoxin cannot reassociate with the inhibitory site on the α-subunit of Na+, K+ ATPase.

Digoxin-specific antibodies are indicated for administration to digoxin toxic patients at high risk of morbidity or mortality. These include patients with:

- Hemodynamic instability ascribed to cardiac glycoside toxicity,
- Dysrhythmias associated with hemodynamic instability,
- Severe bradycardia,
- Altered mental status attributed to digoxin toxicity,
- Hyperkalemia with K+ greater than 5 mEq/L,
- Serum digoxin level greater than 10 ng/mL in adults at steady state

When appropriately used, and in the correct dose, digoxin-specific antibodies can be quite effective in reducing the morbidity and mortality of digoxin toxicity.

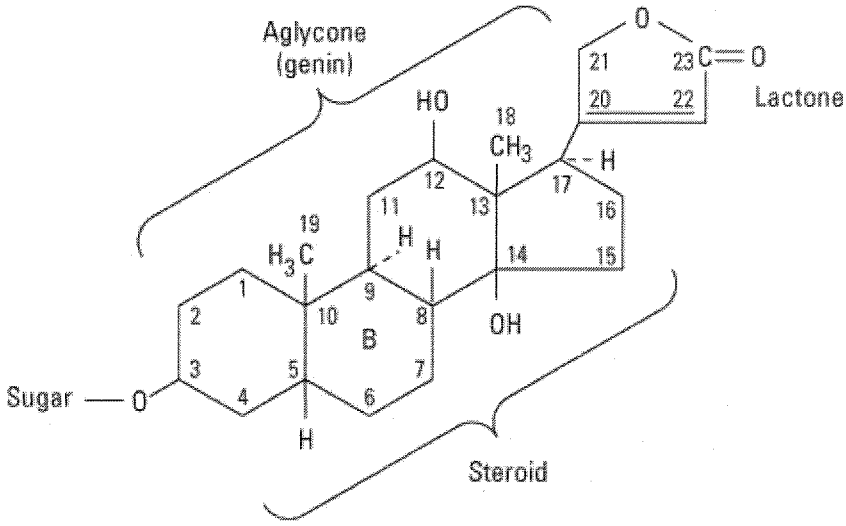
Summary

Digoxin toxicity is a potentially life-threatening condition that because of its narrow toxic-therapeutic window can occur with relatively small increases in plasma concentration. Among its causes are the acute ingestion of a supratherapeutic amount of drug, the chronic administration of drug that is slightly altered in composition or bioavailability, or changes to a patient's concomitant medications or organ function that alter its absorption or elimination. Its therapeutic use in patients with significant cardiac disease is well justified by

current clinical guidelines, however these patients are at greatest risk for adverse outcomes because of their sensitivity to its toxic electrophysiological effects. Prompt recognition and treatment are essential to avoid adverse outcomes. Due to the myriad of signs, symptoms, and electrophysiological abnormalities that can accompany digoxin toxicity, each patient suspected of having digoxin toxicity must be carefully evaluated by a trained clinician.

s/ Dr. Marc J. Semigran, M.D.
Dr. Marc J. Semigran, M.D.
Massachusetts General Hospital
55 Fruit Street
Bigelow 800
Boston, MA 02114

Figure 1. Cardiac glycoside



References

1. Georghaide M, Kirkwood F, Adams, KF et al. Digoxin in the management of cardiovascular disorders. Circulation 2004;109:2959-2964.

2. Lee DC-S, Johnson RA, Bingham JB eta l. Heart failure in outpatients, a randomized trial of digoxin versus placebo.. N Engl J Med 1982;306:699-705.

3. Captopril-Digoxin Multicenter Research Group. Comparative effects of therapy with captopril and digoxin in patients with mild to moderate heart failure . JAMA 1988;259:539-544.

4. Dibianco R, Shabatai R, Kostuk W et al. A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. N Engl J Med 1989;320:677-683.

5. Hunt SA, Abraham WT, Chin WH et al. 2009 Focused Update Incorporated Into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults. J Am Coll Cardiol, 2009; 53:1-90.

6. Preibisz JJ, Butler VP, Lindenbaum J. Digoxin Tablet Bioavailability: Single-Dose and Steady-State Assessment. Ann Intern Med 1974;81:469-474.

7. The Digitalis Investigation Group. The effect of digoxin on mortality and morbidity in patients with heart failure. N Engl J Med 336:525-533

8. Bronstein AC, Spyker DA, Cantilena LR Jr, et al. 2006 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS). Clin Toxicol (Phila). 2007;45:815-917.

9. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Heard SE. 2007 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 25th Annual Report. Clin Toxicol (Phila). 2008;4610:927-1057.

10. Bismuth, C, Gaultier, M, Conso, F, Efthymiou, ML. Hyperkalemia in acute digitalis poisoning: prognostic significance and therapeutic implications. Clin Toxicol 1973; 6:153.

11. Reyes MA, Actor JK, Risin SA, Dasgupta A. Effect of Chinese medicines Chan Su and Lu-Shen-Wan on serum digoxin measurement by Digoxin III, a new digoxin immunoassay. Ther Drug Monit. 2008;30:95-9.